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EFFECT OF ADENOSINE AND INOSINE ON CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN RATS

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Abstract : Liver damage induced in rats by carbon tetrachloride (CCl_4) was obvious macroscopically as well as microscopically in stained sections. Levels of aspartate aminotransferase (AST), alanine aminotransferasa (ALT) and gamma glutamyl transpeptidase (γ-GT) were also significantly raised. Adenosine and inosine effectively countered the damage when these were given before and during the period during which CCl_4 produces the typical damage. The beneficial effect was seen in biochemical as well as pathological studies.

Key words : adenosine, inosine, carbon tetrachloride, liver necrosis, gamma glutamyl transpeptidase

INTRODUCTION

Carbon tetrachloride (CCl_4) induces fatty liver and liver cell necrosis (1). Though the precise mechanism is not known, several effects of CCl_4 seem to play a role such as inhibition of triacylglycerol release from the liver (1) and increase lipoperoxidation in membranes, whose structural integrity is necessary for lipoprotein release, finally resulting in liver triacylglycerol accumulation and destruction of liver cells. Adenosine is a component of the ubiquitous intracellular energy store, ATP, and the intracellular second messenger cyclic AMP. Its presence is associated with inhibition or stimulation of hormones, coronary vasodilation, delayed neurotransmission and changes in the metabolism of fat (2). Adenosine increases the cyclic AMP concentration in lymphocytes, brain slices, vagus nerve, platelets and myocardium while decreases cyclie AMP in fat cells, kidney cortex and liver (2). Adenosine released by the liver appears to play a significant role in replenishing erythrocyte ATP and so keeps the red cell alive (2), and prevent the induction of fatty liver by ethanol and cycloheximide (3). Frederiks (4) reported that adenosine - $MgCl_2$ reduced the extent of necrosis in rat liver subjected to ischemia. The present study was carried out in rats to explore if adenosine and inosine would counteract the biochemical and histological changes associated with CCl_4 induced liver damage in rats.

METHODS

Male albino rats of Wistar strain (HAU, Hissar, 120-140 g) with free access to standard diet (pellets, Hindustan Lever Limited, India) and tap water were used. Animals were divided into 5 groups of 10 each. Group I serverd as control (which received 3 injections of olive oil, 2 ml/kg, ip as vehicle). In Group II fresh mixture of CCl_4 + pure olive oil (1 : 1) was given on the Ist, 4th and 7th day in doses of 2 ml/kg, ip. The group II animals were also administered saline ip (vehicle for nucleosides) for 10 consecutive days. In groups III, IV and V, adenosine (100 mg/kg), inosine (100 mg/kg) and a prepared solution of hydrocortisone sodium succinate (10 mg/kg) respectively, were administered ip in addition to CCl_4. Adenosine, inosine and hydrocortisone solutions were given once daily, beginning one day prior to the experiments and continued for 10 consecutive days. On 10th day blood was withdrawn directly from the heart without using anesthesia and serum was separated by centrifugation for biochemical studies.

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Whole livers were removed after sacrificing the animals and preserved in 10% Formal saline. By the standard technique serial sections (5 μm) were cut and stained with haematoxyline and eosine. Aspartate aminotransferase, AST (EC 2.6.1.1) and alanine aminotransferase, (ALT; EC 2.6.1.2) levels in the serum of different groups were assayed (5). Gamma glutamyl transpeptidase (γ-GT : EC 2.3.2.2.) activity in serum was determined (6).

RESULTS

Liver from control group showed normal appearance, red colour, smooth and regular under surface without any evidence of haemorrhage and necrosis, while CCl_4 treated livers showed multiple area of necrosis with massive haemorrhagic patches. Most of livers were covered with white slough and there were multiple white patches indicating necrotic areas. All livers showed characteristic nutmeg appearance. The under surfaces of most of the livers were irregular/ nodular. Livers from adenosine and inosine treated groups were almost normal in appearance except for mild congestion and minimum fatty changes. Livers from inosine treated group showed less congested areas as compared to adenosine treated group. Under surface of most of the livers from adenosine treated group were nodular, while undersurface of liver was smooth in inosine treated group. Hydrocortisone treated livers were normal in appearance regarding weight, colour and under surface.

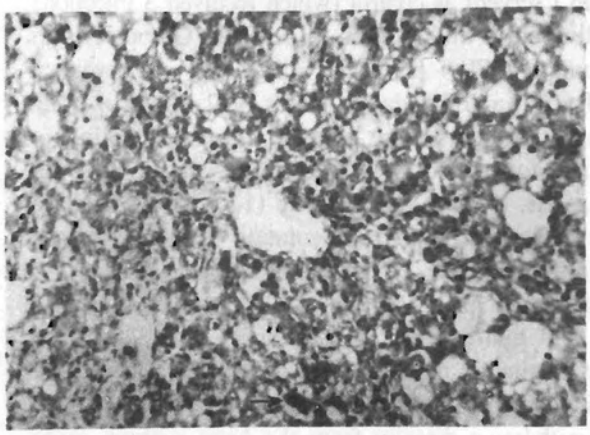

Fig. 1 : Liver Section of CCl_4 treated rat showing intense centrilobular necrosis, haemorrhage (↑) and extensive fatty change (low power : 10 x 10).

Histology of liver from control group showed portal triad, rows of hepatocytes or normal arrangements of hepatocytes with nuclei, while CCl_4 treated liver sections showed intense centrilobular necrosis, sinusoidal haemorrhagic congestion and extensive fatty changes. Hepatocytes in centrilobular zone were enlarged and contained lipids. Hepatocytes in periportal zone were also enlarged and the normal architectural pattern was destroyed with severe vacuolization of surviving periportal hepatocytes (Fig.1). Histology of liver sections of adenosine + CCl_4 - treated rats revealed few areas of congestion, spotty necrosis with minimum fatty changes (Fig. 2).

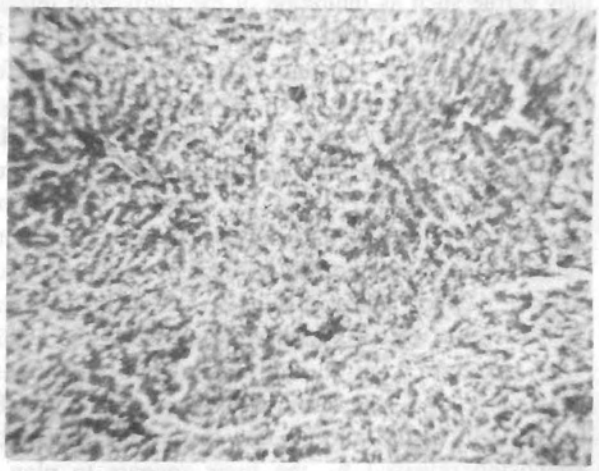

Fig. 2 : Liver Section from adenosine + CCl_4 treated rat showing spotty necrosis with minimum fatty change (10x10).

In inosine + CCl_4 treated group congestion was much less while fatty changes were more than the adenosine + CCl_4 - treated group. In contrast to adenosine + CCl_4 treated group, there was no patches of necrosis in any liver sections from inosine + CCl_4 treated group

(Fig. 3). Hydrocortisone + CCl_4 - treated rat livers showed almost normal appearance except for mild fatty changes (Fig. 4).

Administration of CCl_4 to rats produced a marked elevation of serum AST, ALT and γ-GT levels (Table I). There was a marked reduction in serum AST, ALT and γ-GT levels in rats treated with adenosine + CCl_4, inosine + CCl_4 and hydrocortison + CCl_4 as compared to animals treated CCl_4 alone.

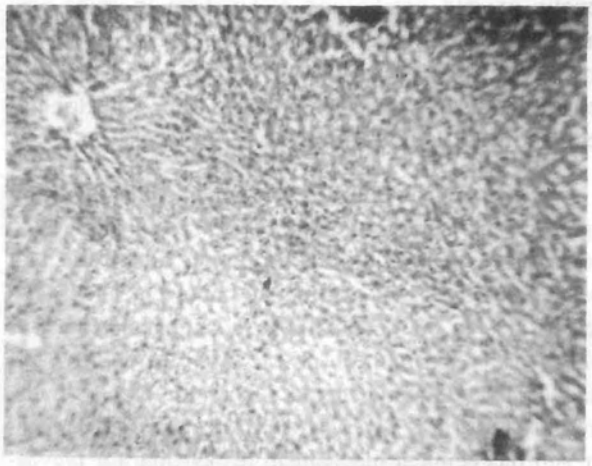

Fig. 3 : Liver section from inosine + CCl_4 treated rat showing mild fatty change (10x10).

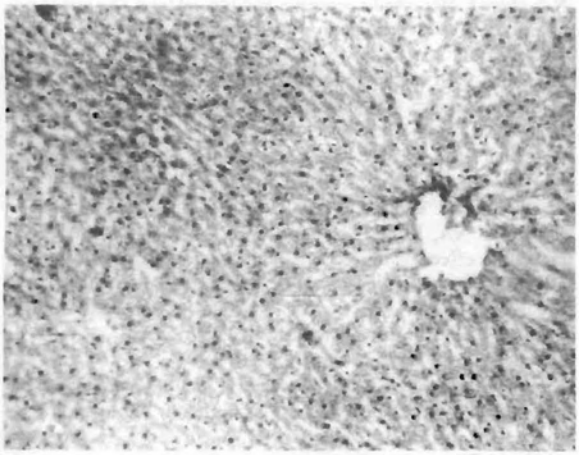

Fig. 4 : Liver Section from hydrocortisone + CCl_4 treated rat showing normal rows of hepatocytes with nuclei and mild fatty change (10x10).

TABLE I : Effect of adenosine and inosine on serum aminotransferase and gamma glutamyl transferase (γ-GT) in rats with CCl_4 induced liver damage, Values are mean ± SEM of 10 animals in each group.

Groups	*Liver Weight (% Body Weight)*	*AST IU/L*	*ALT IU/L*	*γ-GT U/ml*
Control	4.23±0.02	19.2±0.64	16.0±0.39	34.04±1.63
CCl_4	3.75±0.06	52.0±1.46	57.2±2.25	66.91±0.82
Adenosine + CCl_4	4.82±0.08*	24.1±0.32*	28.0±0.51*	45.51±0.31*
Inosine + CCl_4	5.10±0.05*	22.8±0.41*	26.2±0.61*	43.20±0.82*
Hydrocortisone+ CCl_4	4.34+0.03*	20.9+0.28*	23.8±0.46*	39.01±0.41*

*P<0.01 when compared with CCl_4 treated group (Unpaired 't' test)

DISCUSSION

Present study revealed marked protective effect of adenosine and inosine in carbon tetrachloride (CCl_4) induced liver damage in rats. The protective effect of adenosine and inosine may be related to their biochemical and metabolic actions like modulation of extracellular and intracellular Ca^{2+} concentration, providing precursor for ATP synthesis, antilipolytic, antiadrenergic, gluconeogenic, hypothermic and general vasodilator effects. Adenosine is reported to inhibit Ca^{2+} influx into myocardium, vascular smooth muscles and in various parts of brain (7, 8). It is possible that adenosine inhibits Ca^{2+} influx and modulates intracellular calcium which helps in preventing Ca^{2+} accumulation in liver cells, since it was demonstrated that cytosolic Ca^{2+} is elevated 100 folds in rat hepatocytes exposed to CCl_4 which is capable of initiating irriversible liver cell injury (9). It is well known that during myocardial ischemia/infarction break-down products of ATP such as ADP, AMP and adenosine may be neutilized for the synthesis of ATP (10, 11). It is possible that adenosine and inosine increase the ATP production by providing precursor for the synthesis of ATP. Adenosine could diminish the break down of ATP by inhibiting of

the deamination and dephosphorylation of ATP (12). The presence of high concentration of adenosine and inosine can probably act as buffer against diffusion thereby reducing adenine nucleotide degradation (10). Therefore, augmented ATP production and reduced ATP degradation following exogenously administered adenosine and inosine may be responsible for preventing CCl_4 induced lever damage in rats. Since ischemia and ATP depletion are prominent features in CCl_4 poisoning (1).

Well known antilipolytic effect of adenosine and inosine may be beneficial in preventing CCl_4 induced liver damage.

Recknagel (1) proposed that both hepatic necrosis and hepatic lipid accumulation in CCl_4 poisoning were due not to a direct effect of CCl_4 on liver cells but to a massive and persistant discharge of sympathetic nervous system by the action of CCl_4. Adenosine and other nucleotides reduced the release of almost all neurotransmitters whether they are inhibitory or excitatory and antagonize effects of catecholamines in various *in vitro* and *in vivo* preparations (13, 14). Hence, antiadrenergic effect may be beneficial in preventing CCl_4 toxicity.

Strong gluconeogenic effect of adenosine and inosine as reported (2) may be useful in preventing CCl_4 induced liver toxicity. Since liver glycogen is also depleted in CCl_4 poisoning (1).

In addition to biochemical and metabolic actions of these nucleosides, we can not exclude the well known hypothermic and general vasodilator action of adenosine and inosine because hypothermia decreases hepatic oxygen uptake and metabolism in some way and general vasodilatation improves hepatic blood flow, since reduced hepatic blood flow and associated centrilobular hypoxia account for the centrilobular necrosis in CCl_4 poisoning (1).

Biochemical data of the preent study showing significant lowering of serum AST, ALT and γ-GT from their elevated levels following adenosine and inosine administration confirm the involvement of these nucleosides in some important biochemical reactions, which are responsible for the prevention of CCl_4 induced liver damage in rats. From the results of the present study it is difficult to infer the exact molecular and biochemical mechanism responsible for prevention of CCl_4 induced liver damage but the observations suggest marked beneficial effect of adenosine and inosine in liver damage produced by CCl_4.

REFERENCES

1. Recknagel RO. Carbontetrachloride hepatotoxicity. *Pharmac Rev* 1967; 19: 145-208.
2. Fox IH, Kelley WN. The role of adenosine and 2' - Deoxyadenosine in mammalian cells. *An Rev Biochem* 1978; 47: 655-686.
3. Garcia-Sainz JA, Hernan-de-Munoz R, Santamaria A, Pina E, Chagoya-de-Sanchez V. Adenosine prevents induction of fatty liver by Cyclohexamide. *Biochem Pharmac* 1979; 28: 1409-1415.
4. Frederisk WM, Fronik GM. Quantitative analysis of the effect of ATP-$Mgcl_2$ and adenosine-$Mgcl_2$ on the extent of necrosis in rat liver after ischemia. *J Surg Res* 1986;41: 518-523.
5. Reitman S, Frankel S. A colorimetric method for the determination of Serum glutamic oxaloacetic and glutamic pyruvic transminase. *Am J Clin Path* 1957; 28: 56-63.
6. Glick D. In : Interscience publication, Methods of biochemical analysis, Vol. 13, John Wiley & Sons. New York : London 1965; 347-348.
7. Bellardinelli L, Rubio R, Berne RM. Blockade of Ca dependent rat atrial slow action potentials by adenosine and lanthanum. *Pfleugers Arch* 1970; 380: 19-27.
8. Caparrotta L, Fassina G, Froldi G, Poja R. Antagonism between N-6-phenyl Isopropyl adenosine and the calcium channel facilitator Bay K 8644 on guinea pig isolated atria. *Br J Pharmac* 1987; 90: 23-30.
9. Long RM. Moore L. Elevated Cytosolic Calcium in rat hepatocytes exposed to Carbon tetrachloride. *J Pharmac Exp Ther* 1986: 238:186-191.
10. Aussedat J, Vardye SM, Rossi A. Adenine nucleotide synthesis from inosine during normoxic and after ischemia in isolated perfused rat heart. *Cand J Physiol Pharmac* 1986; 63:1159-1164.
11. Silverman NA, Kohler J, Feinberg H, Levistskey S. Beneficial metabolic effect of nucleoside augmentation on reperfusion injury following Cardiopegic arrest. *Chest* 1983 ; 83:787-792.
12. Ely SW. Mentzer RN, Lasley RD, Lee B, Berne RM. Functional and metabolic and reperfusion with adenosine. *J Throc Cardiovas Surg* 1985; 90:549-556.
13. Katims JJ, Murphy KMM, Snyder SH. Xanthine stimulants and adenosine. In : Crease I, eds, Stimulants, Neurochemicals Behaviour and clinical perspective. New York : Revan Press 1983; 63-79.
14. Dobson G. Adenosine reduces Catecholamine contractile response in oxygenated and hypoxic atria. *Am J Physiol* 1983; 245:H 468-474.